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A Continuous-Flow Mini-Diluter System for Toxicity Testing

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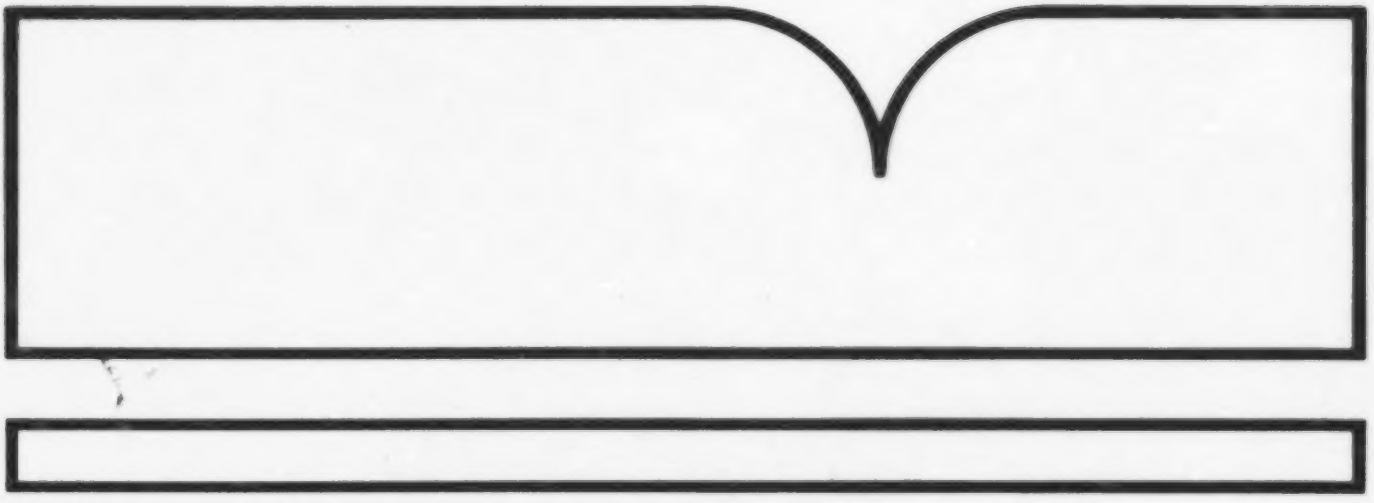

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A CONTINUOUS-FLOW MINI-DILUTER SYSTEM FOR TOXICITY TESTING

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Abstract—A space saving portable mini-diluter exposure system for testing early life stages of fish and invertebrates has been developed and successfully used both in the laboratory and on-site with single chemicals and with complex effluents. This gravity-operated system can also be installed in a compact, vented enclosure to permit safe testing of hazardous volatile chemicals. The mini-diluter test system has several additional advantages over other widely used dosing systems in that it: (1) uses small volumes of complex effluents and/or single chemicals which reduces the problem of removing hazardous material from the test system's waste water; (2) works well with waste containing suspended solids; and (3) can maintain normal operation for up to 10 h if the effluent headbox supply pump fails and up to 4 h if the diluent water headbox supply pump fails.

INTRODUCTION

Increased awareness of water pollution-related problems has prompted the recent development of many reliable and accurate toxicant dosing systems (Lemke *et al.*, 1978; Peltier, 1978). Two of the oldest types of intermittent flow-through systems successfully used were a serial diluter and a proportional diluter described by Mount & Warner (1965) and Mount & Brungs (1967), respectively. Warner (1964), however, was the first to introduce a truly continuous-flow serial diluter (Fig. 1). The flow pattern diagrammed in this figure demonstrates the simplicity of the diluter in which toxicant from one source is continuously diluted by successive additions of water to produce lower and lower concentrations of toxicant. This water delivery system has several advantages over most other dosing systems in that it is gravity operated, does not require critical timing devices, is free of vacuum siphons and/or solenoid valves and once calibrated does not require further adjustment. It is also easily adaptable to a wide variety of uses and applications with either single chemicals or complex effluents. Mean flow deviations are less than 2% at any dilution.

Warner's continuous-flow serial diluter was used in exposure-related trout studies conducted by McKim *et al.* (1970). This system, which worked exceedingly well, was essentially the same as shown in Fig. 1 except that a constant-drip Mariotte bottle, instead of a toxicant pump, was used to meter the chemical solution. During the ensuing years, researchers at the U.S. EPA Environmental Research Laboratories at Duluth, MN and Corvallis, OR used, with increasing frequency, a modified version of the continuous-flow diluter; the modification consisted of a series of baffles, built into the diluter, to ensure adequate mixing of toxicant and dilution water prior to delivery to test tanks. These large scale, single-chemical exposure systems, recently described by Garton (1980), were designed to deliver more than 20 l. test water h^{-1} to each of 5 concentrations plus a control.

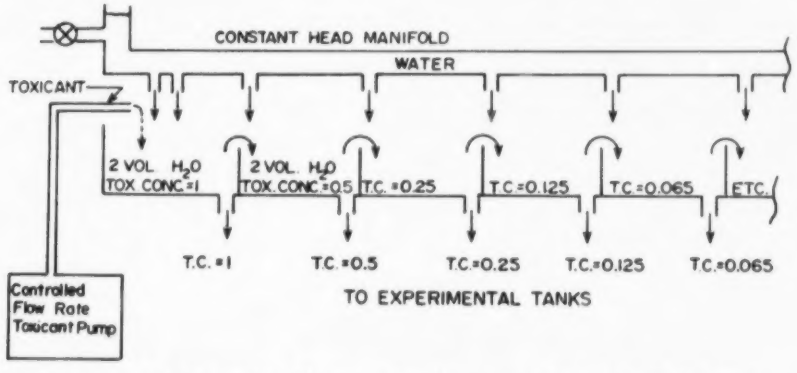

Fig. 1. Dilution principle used in Warner's original continuous-flow serial dilution apparatus (each arrow equals 1 volume of water per unit time).

Interest in developing the early life stage (ELS) fish toxicity test concept introduced by McKim (1977) led to the design of the compact continuous-flow mini-diluter exposure system described in this paper. The system accurately delivers as little as 3 l. test water h^{-1} to each of 5 concentrations plus a control. It can be used in the laboratory or in the field to test the effects of either single chemicals or complex effluents on young fish. The small ELS test apparatus takes very little space and requires only small volumes of test water; this latter point is a critical factor when there is a need to transport effluents to a laboratory test site at some distance. Using smaller volumes of test water also reduces filtration costs when one is required to remove hazardous test chemicals from the diluter waste water before discharge.

The ELS test system described in this paper has been tested and evaluated both in the laboratory and on-site in a mobile trailer. To date, this apparatus has been used to conduct 33 ELS exposures to various toxicants, using the fathead minnow (*Pimephales promelas*) as the test animal. These tests have included metals, pesticides, volatile organic compounds and treated complex effluents from metal plating, oil refinery and sewage treatment plants. The system has also recently been successfully used in tests conducted on macroinvertebrates (Spehar *et al.*, 1980; Anderson, Manuscript).

The following text contains a general description of the mini-diluter exposure system including principles of operation and evaluation. Limited space would not allow us to include detailed construction drawings and photographs. If the reader is interested in further details, complete fabrication instructions can be obtained from the Environmental Research Laboratory-Duluth*.

DESCRIPTION OF CONTINUOUS FLOW MINI-DILUTER

Figure 2 is a photograph of the mini-diluter. The diluter is constructed of double strength glass (3 mm thick) and mounted on a 1.9 cm thick plywood backboard (69 × 38 cm). It is divided into 4 major units as illustrated in the schematic drawing of Fig. 3; these units are the toxicant and water cell (TC and WC), dilution cell (DC); flow booster cell (FBC) and flow splitter cell (FSC).

* Manual for Construction of a Continuous-Flow Mini-Diluter System for Toxicity Testing, by D. A. Benoit *et al.*, U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, MN 55804, U.S.A.

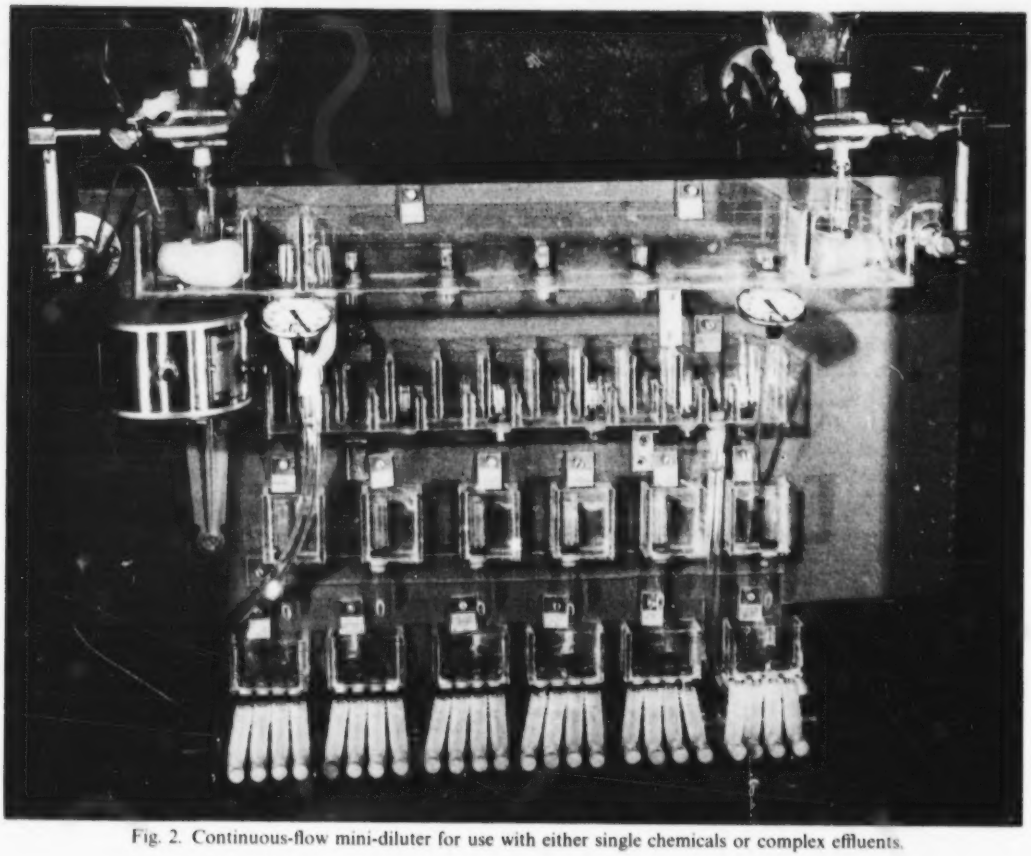

Fig. 2. Continuous-flow mini-diluter for use with either single chemicals or complex effluents.

Toxicant and water cell

The unit containing the constant head toxicant cell (TC) and water cell (WC) measures 7.6 cm wide × 54.6 cm long × 5.1 cm high. Each cell is separated by a vertical glass partition between the emergency outlet (EO) standpipes (Fig. 3). Float valves (FV) which connect to headboxes maintain a constant depth of toxicant or diluent water in each cell and can be adjusted up or down to change head pressure. Emergency outlet drains in each cell are set slightly above the normal water depth. Water from both cells passes through a 20 mesh stainless-steel screen for large particle filtration before it reaches the capillary flow tubes shown in Fig. 3, (T-1 and W-1 through W-5). Capillary flow tubes are initially adjusted up or down in each cell to obtain desired flow rates. Diluter flow rates can also be changed by using different length or diameter flow tubes.

Dilution cell

The second unit of the mini-diluter is a dilution cell (DC) which measures 3.8 cm wide × 37.5 cm long × 5.1 cm high. This cell contains a series of baffles to ensure adequate mixing as the toxicant is continuously diluted by successive additions of diluent water. Principles of operation are diagrammed with arrows shown in Fig. 3. Flow tube T-1 (2 mm, i.d.) delivers toxicant solution (100 ml min^{-1}) to the left end of the dilution cell. Half of this volume, at the rate of 50 ml min^{-1}, flows out the C-1 flow tube (1.5 mm, i.d.) and is the highest test concentration. The other half of the volume, 50 ml min^{-1}, is diluted by one-half when it mixes with 50 ml of diluent water per min from the W-1 flow tube (1.5 mm, i.d.). Half of this combined volume of 100 ml min^{-1} flows out the C-2 flow tube to become the second highest exposure concentration, and the remainder is again diluted by one-half from the next addition of diluent water from the W-2 flow tube. This flow pattern continues until the last dilution is made. The extra volume of test water flows over a baffle and into a water outlet (WO). Water used for the control tank flows through the diluter exactly the same way as the test water. Flow tube W-5 (2 mm, i.d.) delivers control water (100 ml min^{-1}) to the right end of the dilution cell (Fig. 3). Half of this volume, 50 ml min^{-1}, flows out the C-6 flow tube (1.5 mm, i.d.), and the remainder flows over a baffle and into a water outlet.

Flow booster cell

Test water concentrations (C-1 through C-6) flow from the dilution cell to individual flow booster cells (FBC) which measure 2.5 cm wide × 4.5 cm long × 5.4 cm high (Fig. 3). Each of these cells contain one standpipe siphon similar to those described by Benoit & Puglisi (1973). Approximately every 30 s

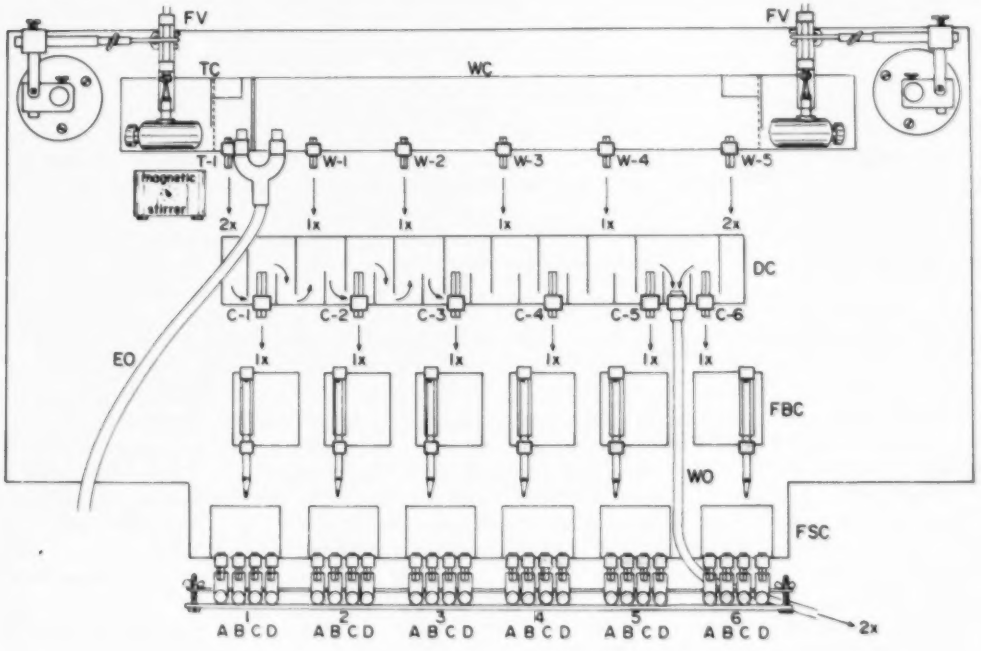

Fig. 3. Schematic drawing and flow pattern of continuous-flow mini-diluter. (C) concentration flow tube; (DC) dilution cell; (EO) emergency outlet; (FBC) flow booster cell; (FSC) flow splitter cell; (FV) float valve; (T) toxicant flow tube; (TC) toxicant cell; (W) water flow tube; (WC) water cell; (WO) water outlet; (1X) one volume; (2X), two volumes.

each cell rapidly empties its content to a flow splitter cell located directly below. As the name implies these flow booster cells are used to increase flow to the flow splitter cells. The rapid filling of each flow splitting cell is necessary to obtain a precise split of the test water through each flow splitting delivery tube.

Flow splitter cell

Flow splitter cells (FSC) measure 2.5 cm wide × 5.7 cm long × 4.8 cm high (Fig. 3). Each cell contains four tubes (1.5 mm, i.d.) cut equally in length and adjusted to the same height. Test water received from each flow booster cell is divided into four equal volumes, thereby creating four replicate concentrations at each treatment level.

Diluter evaluation

Daily flow rate measurements through each of the 12 diluter flow tubes over a 5 week period resulted in an overall mean standard deviation of $\pm 1.3\%$. During a 5 week trial test with dissolved NaCl the mean variability of each concentration ranged from 2 to 7%. In this test, 5 concentrations (0.02–0.3%) were measured daily by conductivity. Variation within each flow splitter cell ranged from 2 to 8%. During four ELS fathead minnow toxicity tests with hexachlorobutadiene, 1,2-dichloropropane, 1,3-dichloropropane and 1,2-dichloroethane, comparisons of chemical concentrations within each group of four replicates, sampled simultaneously, showed that concentrations were within 90% of each other and ranged from 85 to 98% (Benoit *et al.*, 1982).

Float valves are quite sensitive and once they are adjusted to the desired height they will maintain a constant depth of water within ± 2 mm. If headbox pressure changes, the valves will automatically readjust themselves to maintain the same head pressure in the toxicant and water cell. If either float valve sticks open, excess water will flow out the emergency standpipe and the diluter will continue to operate normally.

The mini-diluter will not operate smoothly until the glass flow tubes in each cell wet-up. The system, therefore, should be started at least 24 h before a test is begun. As long as the head pressure remains similar in the toxicant and water cell, no further adjustments are necessary for proper diluter operation during a test except routine cleaning of float valve and flow tube orifices with pipe cleaners. Some test chemicals or effluents can cause fungus to grow very rapidly, in which case diluter flow tubes may have to be cleaned daily. With practice, however, one can complete this task in about 5 min.

All batches of neoprene stoppers should be checked for toxicity prior to their use in the diluter and exposure chambers. Recent static tests have shown that certain lots of neoprene stoppers are acutely toxic to fathead minnow larvae, (Broderius, personal communication)*.

DESCRIPTION OF PORTABLE EXPOSURE SYSTEM

Figure 4 shows a portable early life stage exposure system. The slotted angle iron support frame measures 66 cm wide × 102 cm long × 74 cm high and sits on a 61 × 91 × 66 cm high table. With minor alterations, the same system can be used for testing either complex effluents or single chemicals.

Complex effluent test system

Two identical pair of stainless-steel headboxes are used with the system, one pair to supply diluent water for the diluter water cell and the other pair to provide effluent water for the diluter toxicant cell. Each pair sits on top of the support frame and consists of two interconnected headboxes A and B which are gently aerated to avoid temperature stratification (Fig. 4). Headbox A (30.5 ± 30.5 ± 40.6 cm high) contains a float valve and standpipe drain. If ample quantities of either diluent or effluent water are available, they can be continuously circulated throughout the headbox and out the standpipe. If either supply is limited, float valves can be adjusted so there is no waste overflow. These headboxes also act as a settling basin for large debris and can temper the water several degrees before it is heated to the desired test temperature. Water subsequently flows by gravity from headbox A through a 20 mesh stainless-steel screen for large particle filtration and into headbox B which is insulated and measures 30.5 × 45.7 × 40.6 cm high. The water is then warmed with a 500 W stainless-steel immersion heater and passed through another 20 mesh screen in headbox B before it flows through the diluter to the exposure chambers. Styrafoam covers for either headbox A or B may be used to increase insulation or to reduce the loss of volatile chemicals or effluents (Fig. 4).

Glass exposure chambers are designed to accommodate 15 test fish per chamber and measure 7 cm wide × 18.8 cm long × 9.2 cm high with a water depth of 4.5 cm. Separate delivery tubes distribute the test water from the diluter to each replicate exposure chamber. Delivery tubes are positioned by stratified random assignment. Flow rate to each chamber containing 500 ml of test water, averages 12.5 ml min^{-1} with one chamber volume displacement every 40 min. A white background can be used under the chambers to help make the test fish more visible.

Eggs are incubated in screened (40 mesh) bottom glass jars (120 ml) which slowly oscillate up and down (3.5 cm) in the test water. The glass jars are powered by a 2 rev/min rocker arm assembly (Mount, 1968). One 61 cm long fluorescent tube over the test chambers provides an average light intensity of 20–60 lumens, and photoperiod is controlled by an automatic timer.

* S. J. Broderius, U.S. Environmental Protection Agency, Duluth, MN 55804, U.S.A.

Single chemical test system

In a single chemical test, the headboxes shown in Fig. 4 contain only diluent water which subsequently flows to the diluter water cell and toxiant cell. Predetermined volumes of the test chemical are then delivered to the toxicant cell by an FMI* metering pump and thoroughly mixed with diluent water using a small magnetic stirrer (Figs 2 and 3). Float valves do not have to be readjusted when single chemicals are added directly to the toxicant cell because the valve will automatically readjust itself to maintain the same constant head.

* Fluid Metering, Inc., Oyster Bay, NY 11771, U.S.A. The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

Portable system evaluation

Comparative fathead minnow ELS feeding density tests with clean water were conducted between the small 500 ml chambers described in this report and larger 6000 ml chambers described and used by Benoit & Holcombe (1978). This study was conducted to determine if the smaller chambers with lower flow rates were suitable for normal growth and survival of young fathead minnows. Subsamples of preserved brine shrimp nauplii were counted on a Sedgewick Rafter counting chamber to determine approximate numbers of live shrimp fed. Results of the test, shown in Table 1, demonstrated that although the three feeding densities greatly affected growth, there was no significant ($P = 0.05$) difference in growth or survival between either size of chamber at each feeding density, ($P = 0.05$, analysis of variance with Dunnett's test).

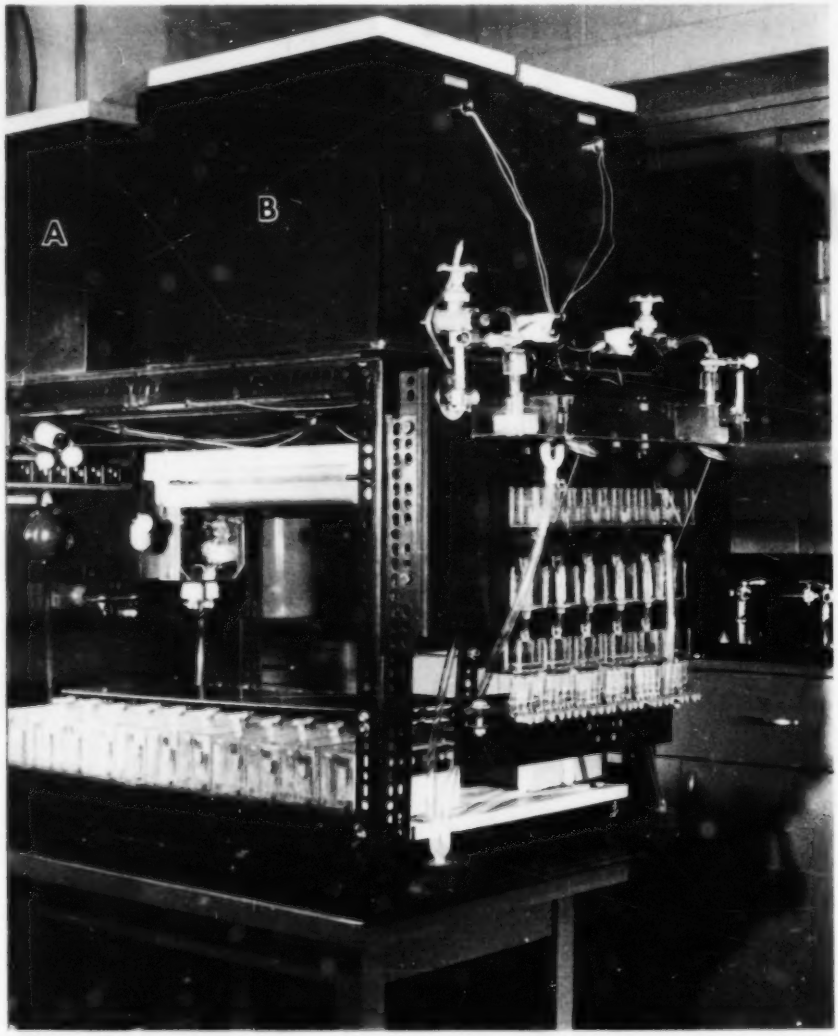

Fig. 4. Portable early life stage exposure system. (A) headbox with float valve; (B) insulated headbox with immersion heater. Water flows by gravity from A to B through an interconnecting pipe.

McKim J. M. (1977) Evaluation of tests with early life stages of fish for predicting long-term toxicity. *J. Fish. Res. Bd Can.* **34**, 1148–1154.

McKim J. M., Christensen G. M. & Hunt E. P. (1970) Changes in the blood of brook trout *Salvelinus fontinalis* after short-term and long-term exposure to copper. *J. Fish. Res. Bd Can.* **27**, 1883–1889.

Mount D. I. (1968) Chronic toxicity of copper to fathead minnows. *Water Res.* **2**, 215–223.

Mount D. I. & Brungs W. A. (1967) A simplified dosing apparatus for fish toxicology studies. *Water Res.* **1**, 21–29.

Mount D. I. & Warner R. E. (1965) A serial dilution apparatus for continuous delivery of various concentrations of materials in water. U.S. Department of Health, Education and Welfare, Health Services Publication 999-WP-23.

Peltier W. (1978) Methods for measuring the acute toxicity of effluents to aquatic organisms. USEPA, Ecology Research Series EPA-600/4-78-012.

Spehar R. L., Tanner D. K. & Gibson J. H. (1980) The effects of kelthane and pydrin on early life stages of fathead minnows (*Pimephales promelas*) and amphipods (*Hyallela azteca*). Presented at the American Society for Testing and Materials 5th Annual Proceedings of Aquatic Toxicology.

Veith G. D. & Comstock V. M. (1975) Apparatus for continuously saturating water with hydrophobic organic chemicals. *J. Fish. Res. Bd Can.* **32**, 1849–1851.

Warner R. E. (1964) Toxicant-induced behavioral and histological pathology: a quantitative study of sublethal toxication in the aquatic environment. U.S. Public Health Service, Contract PH 66-63-72.